

**Connectometry evaluation in patients undergoing carotid
endarterectomy: an exploratory study.**

Keywords

Endarterectomy; DTI; Connectometry.

Abstract

Introduction: Investigating on brain local connectivity changes following Carotid Endarterectomy (CEA) by connectometry.

Methods: In this exploratory study, seventeen subjects (15 males and 2 females, mean age 74.1 years) who underwent CEA, were prospectively recruited. Within one week before the CEA, each patient performed, in the same day, a cognitive evaluation with a Mini Mental State Examination (MMSE) and a Magnetic Resonance (MR) exam that included a DTI sequence for the connectometry analysis. The same cognitive test and MR protocol were performed on follow-up in a period between 3-6 months after CEA. The MMSE scores were analyzed using T-Student test. The connectometry analysis was performed using a multiple regression model in order to consider the effect of CEA, choosing three different T-score threshold values (1, 2 and 3), and results were considered statistically valid when p-value adjusted for False Discovery Rate (p-FDR) < 0.05.

Results: Comparison of pre-CEA and post-CEA MMSE scores showed improvement of MMSE scores after CEA (p-value = 0.0001). Connectometry analysis revealed no areas of statistically significant increased connectivity related to CEA for T-threshold value = 1 and 2, whereas for T-threshold value = 3 the analysis revealed statistically significant increased connectivity after CEA (p-FDR = 0.0106667) in both cerebellar hemispheres and corpus callosum.

Conclusion: The results suggest that CEA procedure is associated with both improvements of cognitive performances and changes in both interhemispheric local connectivity through corpus callosum and in cerebellum.

Introduction

Atherosclerotic disease involving Internal Carotid Artery (ICA) is a well-known risk factor for ischemic stroke [1-3], but also the association between ICA disease and cognitive dysfunctions in patients without clinically evident cerebrovascular disease is well known [4]. In the past years, carotid artery revascularization (following Carotid Endarterectomy (CEA) or Carotid Artery Stenting (CAS)) demonstrated to be protective against occurrence of stroke in symptomatic and asymptomatic subjects [5,6].

Further, several studies investigated on variations in cognitive performance using clinical neuropsychological tests, demonstrating that CAS [7,8] and CEA [9] are associated with increasing in cognitive performances, and these improvements are independent from the treatment type (CEA vs CAS) [10]. Resting State Functional Connectivity Magnetic Resonance (rs-fcMR) allows to study the spontaneous brain networks' activity while patient lies inactive in MR scanner, analyzing Blood Oxygenation Levels Dependent (BOLD) signal fluctuation generated by local changes of deoxyhemoglobin levels of the active brain regions following their activation [11]. DTI allows to evaluate anisotropic diffusion of water molecules inside of the white matter bundles, in order to estimate axonal organization of the brain [12], and tractography allows to study cerebral white matter bundles exploiting DTI principles.

Some of the studies above mentioned analyzed differences in brain connectivity comparing patients with ICA stenosis and normal healthy controls [13-16], others investigated on brain connectivity before and after CAS [17,18], and another one compared changes following CAS or aggressive medical therapy [19]. Another study by *Schaaf et al.* [20] evidenced increased BOLD signal immediately after CEA probably due to the higher intracranial blood flow.

In the last decade, a new tool for the in-vivo analysis of white matter, called "MRI Connectometry", has been introduced in research in order to overcome the limitations of

conventional tractography approach and DTI [21,22]. Connectometry allows to explore the local connectome, i.e. the degree of connectivity between adjacent voxels of the white matter fascicles according to the density of the diffusing spins [23]. The technique is based on the reconstruction of the MRI diffusion data into a standard template space, obtaining a map of the local connectome matrix from a group of subjects. The use of a fiber tracking algorithm on this matrix, both with the comparison of the results with a null distribution of coherent associations using permutation statistics, allows to track only the bundles of white matter that shows a significant positive or negative correlation with the study variable [21]. The spin distribution function (SDF) is the parameter used for the analysis [23]. This method has been already used for example for the study of Parkinson disease [24-30], mood disorders [31,32], multiple sclerosis [33] and amyotrophic lateral sclerosis [34].

The purpose of our study is to identify whether in asymptomatic patients with severe ICA ($\geq 70\%$) stenosis measured according to European Carotid Surgery Trial (ECST) criteria [35] treated with CEA, the cognitive improvements registered in the short-term post-surgical period were also associated with changes in brain local connectivity using MR connectometry, independently from these conditions: a) mono or bilateral severe ($\geq 70\%$) stenosis and b) presence or absence of cognitive dysfunction.

Materials and Methods

Patient enrollment and MR examination technique

The study was designed as a prospective study, and it obtained the approval from the ethical committee. According to the exploratory intent, it was planned to recruit in one year at least 12 patients [36]; from January 2017 up to December 2017 at the University Hospital of Cagliari 17 consecutive patients (15 males and 2 females; age between 65 and 84; mean age: 76.35; mean age

for male group: 75.93; mean age for female group: 79.5) were prospectively enrolled in the study (demographic data reported in [Table 1](#)). All the patients had to be eligible for monolateral or bilateral CEA procedure according to the guidelines of the European Society for Vascular Surgery [\[35\]](#).

The exclusion criteria were the following:

1. Anamnestic history of severe systemic inherited or acquired disease (in particular severe psychiatric/neurological conditions and major stroke), except cognitive dysfunction.
2. Presence of contraindications to MR examinations (e.g. not compatible metal implants).
3. Presence of functional disability, measured with the modified Rankin' scale (values ≥ 2) [\[37\]](#).
4. Symptomatic patients with history of Transient Ischaemic Attack (TIA), Minor stroke or stroke.

All patients recruited signed the informed consent before their enrollment in the study. All patients within the week before the surgical intervention performed the Italian version of Mini Mental State Examination (MMSE) corrected for age and schooling [\[38,39\]](#) for evaluation of cognitive status administered by an expert colleague (DC, 9 years of experience). In the same day a non-contrast resting-state head MR scan was performed with a 1.5 Tesla Philips "Achieva dStream" scanner (Philips, Best, Netherland) (peak amplitude 33mT/m, slew rate 160 mT/m/ms) using a 32 channels head coil. The dedicated MR scan protocol included a DTI sequence with the following parameters: 64 diffusion sampling directions, Echo Time (TE) = 83.147ms, Repetition Time (TR) = 3370 ms, b-values = 0 and 800 s/mm²; in-plane resolution = 1.75 mm, slice thickness 2.5 mm.

The other sequences included in the MR protocol scan were: a) Diffusion Weighted Imaging Single shot (DWI-SSh) sequence, TE = 74 ms, TR = 3546.05 ms, b-values = 0 and 1000 s/mm², slice thickness = 2.5 mm, b) 3D Fluid Attenuated Inversion Recovery (FLAIR), TE = 292.283 ms, TR = 4800 ms, Inversion Time = 1660 ms, Flip Angle 90°, slice thickness = 1 mm, spacing between slices = 0.57 mm.; c) 3D T1-weighted Turbo Field Echo (TFE), TE = 3.43 ms, TR = 7.5 ms, Flip Angle = 8°, slice thickness = 1 mm, spacing between slices = 1 mm.

Patients with MRI findings of acute lacunar and/or territorial stroke, as well as those with imaging evidence of chronic territorial stroke, were excluded from the study; the other exclusion criteria were the presence of incidental findings suggestive for intra-axial or extra-axial neoplastic lesions, inflammatory or infective diseases. On the contrary, patients with small hyperintense areas on FLAIR sequences suggestive for leukoaraiosis/chronic lacunar strokes were included in the study, due to the fact that usually they are common findings in patients with ICA severe ($\geq 70\%$) stenosis and often clinically asymptomatic [40] and the study was designed as longitudinal. No surgical neither post-surgical complications occurred to any patients during hospitalization.

Follow-up MMSE and MR examination, with the same sequences and parameters used in the first MR evaluation, were performed in the same day after a period between 3 and 6 months from the surgical intervention (average follow-up time: 4.08 months).

Connectometry analysis

Regarding the analysis of imaging investigations, a total of 34 diffusion MRI scans (17 pre-CEA and 17 post-CEA) were included in connectometry database. The analysis of the data was conducted using DSI Studio (release 2017_08 - <http://dsi-studio.labsolver.org>). The diffusion data were reconstructed in the Montreal Neurological Institute (MNI) space using q-space diffeomorphic reconstruction [23] to obtain the SDF [41], adopting the Human Connectome Project 1021 (HCP-

1021) template as diffusion MRI atlas [23]. A diffusion sampling length ratio of 1.25 was used, and the output resolution was 1 mm. The restricted diffusion was quantified using restricted diffusion imaging [42]. The SDF values were used in the connectometry analysis.

Diffusion MRI connectometry [21] was used to study the effect of CEA. CEA variable was associated with this local connectome matrix in order to identify those local connectomes that expressed significant associations with it.

Three different T-score threshold values (1, 2 and 3) were assigned in three consecutive analyses in order to select local connectomes, and the local connectomes were tracked along the core pathway of a fiber bundle using a deterministic fiber tracking algorithm and compared with a null distribution of coherent associations using permutation statistics [21,43,44]. Track trimming was conducted with 1 iteration. All tracks generated from bootstrap resampling were included. A length threshold of 35 mm was used to select tracks. The seeding density was 40 seeds per mm³ and a total of 6000 randomized permutations were applied to the group label to obtain the null distribution of the track length. These parameters were chosen in order to improve specificity of the analysis and trying to overcome the limits due to the low number of available cases (even if the research was designed as exploratory).

Statistical analysis

Statistical analysis was performed with the SPSS 24.0 statistical package (SPSS Inc, Chicago, IL). The normality of each continuous variable group was tested using the Kolmogorov-Smirnov normality test. The pre-CEA and post-CEA MMSE scores then were compared using T-Student for paired values test, and a p-value < 0.05 was regarded to indicate statistically significant association. All p-values were calculated using two-tailed significance level.

The connectometry analysis used a multiple regression model to evaluate CEA variable using three different T-score threshold values (1, 2 and 3). Permutation test allowed to estimate and correct the false discovery rate (FDR) of Type-I error inflation due to multiple comparisons [21]. p-value corrected for FDR (p-FDR) < 0.05 was regarded to indicate statistically significant association.

Results

None of the subject suffered surgical or medical complications after the procedure. The analysis of follow-up structural MR sequences after CEA did not reveal any new incidental findings, included territorial or lacunar strokes.

The analysis of MMSE examinations revealed that after CEA procedure all the patients improved their cognitive performance (p-value = 0.0001), with Pre-CEA mean average score of 19.03 and Post-CEA mean average score of 23.82 (Figure 1 and 2).

The connectometry analyses performed using T-score threshold = 1 and 2 did not reveal statistically significant results. The same analysis performed adopting T-score threshold value = 3 showed greater local connectivity after CEA procedure in both the cerebellar hemispheres and corpus callosum (p-FDR = 0.0106667) and no tracts with decreased local connectivity (Figure 3 and 4). The mean SDF value of each subject for corpus callosum and cerebellar hemispheres were also reported (Table 2).

Discussion

The correlation between carotid artery stenosis, cognitive function and brain connectivity impairments was described in previous researches. For example, it is known that both CAS and CEA are associated with long-term improvements in cognitive performances [45].

Different MR studies tried to identify brain structural, functional and biochemical differences among patients with asymptomatic ICA stenosis and HCs; in particular *Lin CJ et al.* [13] compared a cohort of 30 cognitively intact subject with asymptomatic, severe ($\geq 70\%$) unilateral ICA stenosis and a second cohort of healthy controls (HCs) using DTI, resting state fcMR, and a comprehensive battery of neuropsychiatric tests: on DTI that the whole brain Fractional Anisotropy (FA), an index supposed to be directly correlated to the degree of myelination of white matter fibers [46], was reduced in patients with asymptomatic unilateral ICA stenosis than HCs; on resting state fcMR the cohort of asymptomatic patients showed reduced connectivity of the Default Mode Network (DMN), Dorsal Attention Network (DAN), fronto-parietal network and sensorimotor network. A similar approach was adopted by *Chang TY et al.* [14], comparing patients with unilateral ICA stenosis ($\geq 60\%$) and HCs using a battery of neuropsychological tests, resting state fcMR and perfusion MR. Network analysis revealed that in the patients' group the hemispheres ipsilateral to the ICA stenosis was impaired in "degree" and "global efficiency". *Avirame K et al.* [15] suggested that cerebral vascular autoregulation, in terms of vasomotor reactivity measured by transcranial Doppler, can be one of the mechanisms involved in structural and functional connectivity impairment of cerebral networks in asymptomatic patients with either ICA occlusion or high-grade ICA stenosis. A research by *Wang T et al.* [16] compared patients with asymptomatic ICA stenosis and HCs using cognitive tests and an integrated MR approach that consisted of pulsed Arterial Spin Labeling (pASL), proton spectroscopy (MRS) and resting state fcMR: this research revealed that the condition of ICA stenosis was associated with lower scores at the neurocognitive tests and decreased cerebral blood flow (CBF) in left frontal gyrus, decreased N-Acetyl-Aspartate (NAA) / Creatine (Cr) ratio in the left hippocampus and reduced connectivity in PCC and anterior part of the DMN.

Few longitudinal studies investigated on the effects of ICA revascularization. In 2010 *Schaaf M. et al.* [20] studied the early effects of CEA on functional MR, evidencing that immediately after CEA BOLD signal changes as reflection of ameliorated cerebrovascular reactivity, but in this paper the only mean signal-intensity change was assessed. *Cheng HL et al.* [17] compared HCs with patients with $\geq 70\%$ asymptomatic stenosis of unilateral internal carotid artery using a comprehensive neuropsychological battery and a multimodality neuroimaging approach, that included DTI and fcMR; patients with carotid artery stenosis showed poorer performances in cognitive tests and marked reduction of inter-hemispheric and intra-hemispheric connectivity ipsilateral to carotid stenosis at the level of the fronto-parietal DMN; after successful CAS, small but measurable increments of the mean FA and functional connectivity in the DMN regions were noted. Recently *Wang T et al.* [18] demonstrated on fcMR that cognitive improvements observed after CAS in asymptomatic patients can be partly correlated with increased connectivity to the posterior cingulate cortex (PCC) in the right SupraFrontal Gyrus (rSFG) on resting state functional connectivity Magnetic Resonance (fcMR) and increased perfusion in the left frontal gyrus (IFG) pulsed Arterial Spin Labeling (pASL) that underwent CAS procedure. To our knowledge, there are not similar studies that investigated brain networks activity changes on fcMR after ECA in patients with severe ICA stenosis. Another study by *Lin CJ et al.* [19] analyzed longitudinally two different groups of asymptomatic patients with severe unilateral ICA stenosis ($\geq 70\%$) by using neuropsychological tests, structural MR imaging, DTI and resting state fcMR: one group was treated with aggressive medical therapy alone and the other one with aggressive medical therapy in combination with CAS. Patients were evaluated at the baseline and three months after treatment: they found that group treated with aggressive medical therapy and CAS showed a small increase in FA at the splenium of the corpus callosum, an increase functional connectivity of the DAN at the level of insular cortex and of the DMN at the level of MPFC.

In this analysis, we investigated if there were short-term brain local connectivity changes after CEA, and if they were accompanied or not by changes in cognitive performances measured by MMSE. The group connectometry analysis revealed that patients who underwent CEA showed statistically significant ($p\text{-FDR} = 0.0106667$) short term local connectivity changes in corpus callosum and cerebellum. a statistically significant greater connectivity when compared to the baseline; no areas of reduced local connectivity were found. The Pre-CEA and Post-CEA MMSE scores analysis showed a statistically significant improvement of MMSE scores after CEA ($p\text{-value} = 0.0001$), with Pre-CEA mean average score of 19.03 and Post-CEA mean average score of 23.82.

To the best of our knowledge, this is the first longitudinal study that used the connectometry technique in order investigate on the mid-term local connectivity changes after ICA treatment. Differently from fcMR that analyzes the spontaneous brain networks' activity of local brain areas (in particular at the level of grey matter) exploiting the BOLD signal differences, the connectometry technique allows to analyze the local connectome, i.e. to track the local connectivity patterns along the WM fibers pathways associated with a determine study variable, in this case CEA procedure.

As indicated above, previously published studies has investigated connectometry differences in other neurological diseases [24-34], adopting SDF as biomarker of investigation. SDF is a density-based measurement of diffusion at different orientations and it measures the density of diffusing water, differently from other diffusivity measurements such as Fractional Anisotropy (FA), Apparent Diffusion Coefficient (ADC) and Radial Diffusivity. The reproducibility and uniqueness of SDF is higher than other diffusivity-based measurements: a paper by *Yeh F-C et al.* [47] in fact showed that SDF provides a unique structural characterization that can reliably identify single subjects (local connectome fingerprint). Since SDF reveals high individuality, it can be considered a good parameter of inter-subject variance and connectometry can be considered suitable for longitudinal study. We adopted this analysis technique also because differently from other track-based or region-based

diffusion analysis that compare diffusion data within a given region, connectometry technique is able to track the differences in the whole brain.

The results of our connectometry analysis reveal that following CEA procedure, corpus callosum and both the cerebellar hemispheres show incremented local connectivity.

The study by *Cheng TL et al.* [17], reported above, evidenced that asymptomatic ICA stenosis is associated with reduced interhemispheric connectivity, and it is known that corpus callosum is implied in interhemispheric communication [48,49]. We can speculate that the augmented local connectivity reflects incremented interactions between cerebral hemispheres.

Regarding the cerebellar findings, it is noteworthy the fact that the proofs on the role of cerebellum in cognitive functions are growing up [50,51]. From an evolutionistic point of view, the expansion of the prefrontal region of the brain is associated with volume expansion of cerebellar regions Crus I and II [52]; further it is known that cerebellar regions Crus I and II are directly interconnected with prefrontal regions and participate to cognitive processes [53] and it is also known that cerebellar changes are not only largely disease-specific, but they are also associated with cortical and subcortical changes in neurodegenerative conditions [54]. Also in this case, the incremented local connectivity of the cerebellar hemispheres following CEA could be implied in the improvement of neurocognitive performances.

The low number of longitudinal studies on the effects of ICA revascularization, the absence of a complete knowledge of the mechanisms underlying neurocognition in normal healthy subjects, and the relatively recent introduction of the connectometry technique for the study of neurological diseases limit the interpretation of the results, and further similar investigations on healthy and diseased subjects need to be done.

Anyway, to our knowledge, this research both with other similar researches that analyze the effects of carotid revascularization on cognitive performances and cerebral networks re-

arrangements, could represent starting points to re-think the idea of carotid revascularization not only for prevention of stroke, but also for therapeutic intents in selected patients with cognitive impairment.

In this study there are some limitations. The first one is the small number of patients: our main focus was to explore the potential role of connectometry technique in the study of brain variation after CEA in patient with severe carotid artery stenosis; also with the small cohort considered, our analysis gave statistically significant values ($p\text{-FDR} = 0.0106667$) that allows us to confirm the validity and the strength of this model and results. However further studies with bigger cohort are necessary to further expand the model by including more variables such as the mono/bilateral severe stenosis and or presence/absence of cognitive dysfunction.

Another limit is the use of MMSE as the only test used for the evaluation of the cognitive functions of the patients: cognitive improvements seen after CEA were already demonstrated in previous researches that patients who underwent CEA showed improvements in cognition, mood and quality of life tests [8,45]. Even if MMSE is not the most appropriate test for cognitive analysis in patients with ICA stenosis, it was used as surrogate test in order to give general indications on the trend of neurocognitive performances before and after the surgical procedure due the exploratory intent of the study.

MR technique adopted could represent another limit; the majority of connectometry study have been performed using 3.0 Tesla MR scanners and b-values higher than 800 mm/s^2 ; our intent was to investigate on the potential role of the connectometry technique also in everyday clinical practice, using relative fast sequences easy to be included in a standard MR scan protocol, and this is also the reason why we did not perform a fcMR study on the same patients.

Conclusions

This exploratory research investigated on the short-term effects of CEA on neurocognitive performances and brain using connectometry in asymptomatic patients eligible for CEA. The results obtained suggest that the cognitive improvement observed can be related to increased local interhemispheric connectivity cerebellar local connectivity, independently from the cognitive status and the condition of mono or bilateral ICA stenosis.

Conflict of interest statement

We declare that we have no conflict of interest.

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Table Legend

Table 1: Demographic data.

Table 2: individual mean sdf values of corpus callosum, right and left cerebellum.

Figures Legends

Figure 1: Graphs and resume table of the Kolmogorov-Smirnov test that confirmed the normal distribution of both Pre-CEA and Post-CEA MMSE scores. df = degrees of freedom; Sig. = significance.

Figure 2: Graphs and resume table of the T-Student for paired values test, showing improvement of MMSE scores after CEA (p-value = 0.0001), with a Pre-CEA mean average score of 19.03 and Post-CEA mean average score of 23.82. N = number of samples; Std deviation = Standard deviation; Std Error mean = Standard error of the mean; t = t-value; df = degrees of freedom.

Figure 3: Brain regions that showed increased local connectivity after CEA procedure. The color of the tract depends on the direction of the fibers (red for right-left, blue for foot-head, green for anterior-posterior).

Figure 4: p-FDR trend (vertical axis) in relation to fiber length (horizontal axis) for T-Score threshold = 3.